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# HAEMOGLOBINAEMIA IN FRESH WATER FISH, HETEROPNEUSTES FOSSILIS DUE TO TOXICITY OF FERTILIZER UREA

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**ABSTRACT** Decrease of Biological Oxygen Demand (BOD) of water due to Toxicity of urea fertilizer resulted in lowering of heaemoglobin in fresh water fishes *Heteropneustes fossilis* at six different concentrations (8.60 gm/1 to 36.70 gm/1) and interval of exposures of 144 to 24 hours, The fishes tried to adjust themselves and marked behavioral changes were observed.

**KEYWORDS :** Urea, Fertilizer, *Heteropneustes fossilis*, haemoglobin.

## INTRODUCTION

In freshwater fishes a large number of workers have studied the haematological parameters in natural ecological conditions as well as in the experimentally created aquaria with contaminants under controlled laboratory conditions (1,2,3,4 & 5). These workers concluded from their experiments that aquatic pollutants in the medium, induce marked hematological alterations and hence fish blood parameters can be used as indicators or monitors of prevailing environmental pollution. Urea fertilizer is commonly used in agricultural practice to increase crop yield. Effect of urea fertilizer, (Nitrogen 45% purchased from Government approved Agricultural Shops, from Daliganj, Lucknow) on Haemoglobin, of teleost fish *Heteropneustes fossilis* exposed for 24 to 144 hours, to six different concentrations (8.60 gm/1 to 36.70 gm/1), was studied and results are interpreted in this paper in the background of aquatic pollution.

## MATERIALAND METHODS

Healthy and live fishes *(Heteropneustes fossilis)* were obtained from the Gomti River at Lucknow through fish Catchers. Fishes were brought to laboratory in wide mouthed glass bottles in natural water, and washed 3 times in tap water, then treated with 2.5% $KMnO_4$ to remove infections like protozons, fungi, bacteria, trematodes, arthropods etc. Apparently normal, uninfected and only healthy fishes, were transferred to large glass aquaria. Fishes, were acclimatized for 12 hours. Earlier, the lethal concentrations of urea fertilizer for particular period i.e. for 24, 48, 72, 96,120,144 hours were recorded (6,7).

Water characteristics were analysed before and during the experiment following the standard procedure. Proper oxygen supply was maintained throughout the experiment. Fishes were taken out after definite interval and exposed to desired concentration of the fertilizer, avoiding injuries and stresses of all kinds as for as possible. Blood was collected from the live fish (by puncturing the caudal vein) in vials and mixed with anticoagulant Ethylene Diamino Tetra Acetic acid (EDTA). Data in respect of Haemoglobin (gm%) were obtained using Boehringer Mannheim Diagnostic HG-555.

## OBSERVATIONS AND RESULTS

### 1. Water Characteristics

Variable changes in water characteristics were observed. (Table - 1). After dissolving the fertilizer, pH of the medium, slightly changed and the other water quality (11) remain almost unaffected.

### 2. Behavioural Changes

At lower concentrations i.e. 8.60gm/l to 21.45gm/l, the fishes showed sluggishness, swimming slowly on aquaria corners, and many tried to come out of aquaria, in 72 hours. In mean exposure i.e. at 27.90 gm/1, the fishes were almost restless and gathered on one corner. The fishes appeared gasping, became sluggish, and tried to engulf air and water with much more speed. At higher concentrations i.e. 31.95 gm/1 and 36.70 gm/1, fishes turned upside down in position soon after the exposure and they died in comparatively shorter intervals. In water, mucus secretions were seen in abundance after 48-72 hours of exposure.

### 3. Hematological Changes

The Haemoglobin (Table-II) decreased at all concentrations and exposures. At lower concentrations, after shorter intervals slight decreases were seen, while at higher concentrations, significant decrease in shorter intervals were observed and concentrations proved lethal to fishes. Maximum lowering of 82.44% in Haemoglobin content was recorded.

**Table- I**

**Showing water characteristics after dissolving urea fertilizer at different intervals**

| Water Characteristic | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fertilizer concentration (gm/1) | Exposure time in hours | Temperatue (Aquaria) 0C | pH | Hardness (Total p.p.m.) | Hardness (temporary) | Alkalinity (pernopthaline) p.p.m. | Alkalinity (Methylorange) p.p.m. | Alkalinity (Methyloran ge) p.p.m. | Dissolved Oxygen p.p.m. |
| * Control | *100<br>*144 | *36.5<br>*35.9 | *29.0<br>*28.5 | *7.05<br>*7.05 | *244<br>*255 | *244<br>*253 | *15.0<br>*10.0 | *245<br>*255 | *9.2<br>*8.9 |
| 8.60 | 00<br>144 | 36.5<br>35.7 | 28.0<br>28.5 | 8.02<br>9.05 | 240<br>244 | 240<br>224 | 20.0<br>162.5 | 305<br>412.5 | 9.0<br>8.7 |
| 12.25 | 00<br>120 | 36.5<br>36.2 | 27.5<br>28.0 | 8.10<br>8.65 | 236<br>227 | 236<br>227 | 25.0<br>155.0 | 324.5<br>402.0 | 9.1<br>8.8 |
| 21.45 | 00<br>96 | 36.5<br>36.0 | 27.0<br>28.0 | 8.12<br>8.57 | 235<br>223 | 235<br>223 | 40.5<br>125.0 | 365.5<br>435.0 | 8.9<br>8.6 |
| 27.80 | 00<br>72 | 36.5<br>36.2 | 26.5<br>28.0 | 8.15<br>8.56 | 232<br>239 | 232<br>219 | 85.0<br>49.5 | 381.5<br>446.0 | 9.0<br>8.4 |
| 31.95 | 00<br>40 | 36.5<br>36.0 | 26.0<br>27.5 | 8.21<br>8.84 | 214<br>196 | 214<br>196 | 121.5<br>85.0 | 403.0<br>454.5 | 8.9<br>8.2 |
| 36.70 | 00<br>24 | 36.5<br>36.7 | 24.0<br>26.5 | 8.24<br>8.93 | 209<br>187 | 209<br>187 | 140.0<br>64.5 | 423.5<br>467.0 | 9.1<br>8.5 |

* Control values

**Table-II**

**Effect of fertilizer on Hb (gm%) levels of fresh water teleost Heteropneustes fossils.**

| Fertilizer Contration gm/l No. of observation 10 in each case | Hb (gm%) Mean+ S.D. (Range in Parenthesis) Exposure Time in Hours | | | | | |
|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 | 144 |
| | | | Control value | 6.87 ± 0.38 (6.50-7.90) | | |
| 8.60 | 6.00±0.18 (5.80-6.20) | 5.37±0.20 (5.20-5.60) | 4.67±0.26 (4.30-4.90) | 4.05±0.12 (3.90-4.200) | 3.35±0.0.38 (2.90-3.800) | 2.57±0.22 (2.30-2.80) |
| 12.25 | 5.55±0.35 (5.20-5.90) | 4.47±0.40 (4.00-4.90) | 3.40±0.42 (2.90-3.80) | 2.82±0.49 (2.30-3.50) | 2.22±0.17 (2.00-2.40) | |
| 21.45 | 5.02±0.22 (4.80-5.30) | 4.32±0.28 (4.00-4.70) | 3.55±0.42 (3.00-4.00) | 2.45±0.42 (2.00-2.90) | | |
| 27.80 | 3.00±0.35 (2.70-3.50) | 2.04±0.21 (2.20-2.60) | 2.25±0.26 (1.90-2.50) | | | |
| 31.95 | 2.60±0.49 (1.90-3.00) | 1.72±0.17 (1.50-1.90) | | | | |
| 36.70 | 1.22±0.22 (1.00-1.50) | | | | | |

## DISCUSSION

It is an experimentally established fact that various pollution in the water medium as well as those injected into the fish, result into alterations of haematological parameters. A sublethal dose of 10 ppm of Rogor, on organophosphorus pesticide, resulted in an increase of leucocytes in *Clarias batrachus* from 25.55 thousand per c. mm to 32.00 thousand per c. mm in 96 hours (2) Carbon tetrachloride (injected 0.03) ml and 0.06 ml per 100 gram body weight at intervals of 3 days) caused considerable lymphocytosis i.e. in a period of six days, lymphocytes increased from 33% to 72%. At 10 ppm Rogor resulted in a fall of total RBC count in *Clarias batrachus* from 3.85 to 2.21 millions/c mm in 96 hours (2). While total RBC count decreased from 5.05 to 2.70 millions/c mm in six days in $CCl_4$ injections . A fall in RBC count from 2.91 to 1.73 millions/c. mm in 10 days was observed in endosulfan poisoning (4). In *Heteropneustes fossilis* pesticide Malathion at 7.6 ppm resulted in a fall of RBC Count from 6.40 to 3.46 millions per c mm in 96 hours (7).

In *Clarias batrachus*, haemoglobin content decreased from 15.34 gm% to 10.30 gm% in 96 hours at 10 ppm or Rogor (2), 10.03 gm% to 8.60 gm% in six days in $CCl_4$ injections 13.05 gm% to 9.13 gm% at 0.01 ppm of endosulfan (4), while in *H. fossilis* 7.6 ppm of malathion in the medium resulted in a fall of heamoglobin count from 12.35 gm% to 11.42 gm% , Urea toxicity syndromes in fishes may be considered due to its nitrification and decomposition to $NH_3$ in soils and waters. Urea, commonly used as fertilizer in India, leaches out in water areas and persists there in larger quantities than used in this study and the toxicity of urea fertilizer was evident in our observations.

In *Tilapia guinensis* various experiments were done using organic fertilizers (9). Histopathological changes due to the use of methiocrab or endosulfan were studied on ranbow trout (8). Sub lethal dose of ammonia and urea concentrations inhibited physiological disorder in rainbow trout *Oncorhynchus mykiss,* (10), In the same fish ammonia and urea affected the activity of enzyme, gillcarbonic anhydrase (12), The tobacco leaf dust altered the biochemical parameters of Hybrid catfish, *Clarias gariepinus* and *Heterobranchus bidorsalis* (13). The organophosphate (Dimethoate) caused Acute Toxicity and Behavioral Responses of Common Carp *Cyprinus carpio*, (14). In fishes ammonia toxicity caused many physiological disorders (15). Acute toxicity of inorganic fertilizers to African catfish, *Clarias gariepinus* (Teugals) had also been reported (16). Acute toxicity levels and ethological responses were reported in *Channa striatus* to fertilizer and industrial wastewater (17). Toxicity in rice-cum-fish culture systems were reported due to the effect of use of different fertilizers (18). Fertilizers and drainage from turf grass affected the quality of ground and surface water (19). Chronic ammonia toxicity to duckweed fed Tilapia, *Oreochromis niloticus* had also been reported (20). Haematological response of African catfish *(Clarias gariepinus)* & rat to crude oil exposure had also been reported (21).

The above findings lead to fall in haemoglobin, in fresh blood are common effect of pollutants in the aquatic environment.

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